PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Andreas MEINKE *et al.*

Serial No.: 10/556,060

Filed: 11/07/2005

For: S. AGALACTIAE ANTIGENS I + II

Group Art Unit: 1645

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CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this Declaration is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

May 14, 2007 — Date
Travis M. Wohlers

DECLARATION OF BEATRICE SENN Ph.D. UNDER 37 C.F.R. § 1.132

I, Beatrice Senn, hereby declare as follows:

1. I am an Swiss citizen residing in Vienna, Austria. I am the Head of Infectious Disease Models at Intercell AG. I have extensive research experience in the field of immunology as evidenced by the publications listed in my *curriculum vitae*. A copy of my *curriculum vitae* is attached as Exhibit 1.

2. I have reviewed the specification of the above-reference application, the amended set of claims, and the Office Action dated December 13, 2006 ("the Action"). I understand that the Action rejected certain claims for lack of enablement. I am submitting this declaration to provide additional data demonstrating that a fragment of the *S. agalactiae* antigen gbs2018 is capable of

3.The gbs2018 antigen used in the studies described in this declaration is the same as the gbs2018 antigen described in the present patent specification and designated as SEQ ID NO: 364 (Specification, Table 1A, p. 77). For the studies described in this declaration, a fragment of the gbs2018 antigen corresponding to amino acids 36 to 612 of SEQ ID NO: 364 was used. The gbs2018 gene was amplified for genomic DNA of *S. agalactiae* ATCC12403 (serotype III) by PCR using gene specific primers. The primers also contained restriction sites that aided in a directional cloning of the amplified PCR product. The PCR products were digested with the appropriate restriction enzymes and cloned into the pET28b (+) vector (Novagen) for His-tagged proteins. Once the recombinant plasmid was confirmed to contain the gene of interest, the plasmid was transformed into *E. coli* BL21 Star® cells (Invitrogen). For the expression of gbs2018 protein antigen, *E. coli* BL21 Star® cells harboring the plasmid were grown to log phase. Once an OD_{600} of 0.6 was reached, the culture was induced with 0.5 mM IPTG for 3 hours at 37° C. The cells were harvested by centrifugation, lysed by a combination of the freeze-thaw method followed by disruption of cells with Bug-buster® (Novagen). The hexa His-tagged gbs2018 protein was then purified on a nickel affinity column. The isolated protein was then assayed by Bradford for protein concentration and checked by SDS-PAGE and Western blot.

4.Polyclonal rabbit sera were generated for gbs2018 and gbs0031 at Charles River Laboratories, Kislegg, Germany. Gbs0031 is a known protective protein antigen. The gbs0031 antigen was also used as a positive control in the studies described in the specification (*e.g.*, Example 6). Animals were pre-screened for pre-existing GBS-specific antibodies by testing their sera with ELISA and only animals without a significant reaction were used. 250 µg of recombinant protein adjuvanted with Complete Freund adjuvant (CFA) was injected into New Zealand White rabbits. Animals were boosted three times with the same amount of protein, but with Incomplete Freund

adjuvant (IFA), at days 28, 42, and 56. Antibody titers were measured at day 38 and 52 by ELISA. Rabbits were terminally bled at day 70.

5.For passive immunization studies, CD-1 mice were immunized intraperitoneally with 150 μl of the gbs2018 or gbs0031 rabbit hyperimmune sera 1 to 3 hours before the bacterial challenge. As a negative control, mice were immunized with 150 μl of phosphate buffered saline (PBS). Animals were pre-screened for pre-existing GBS-specific antibodies by testing their sera with ELISA and only animals without a significant reaction were used in the study. Freshly grown *S. agalactiae* strains C388/90 (serotype Ia), A909 (serotype Ia), ATCC12401 (serotype Ib), ATCC12403 (serotype III), COH1 (serotype III), ATCCBAA22 (serotype III), 2603V/R (serotype V), ATCC49447 (serotype V), and ATCCBAA23 (serotype V) were used for the animal challenge studies. In order to determine the viable cell numbers present in the bacterial inoculum, colony forming units (cfus) were determined by plating on blood agar plates. 10^6 – 10^8 cfus were applied intraperitoneally into mice. Protection was measured by a lethal sepsis model, where survival rates were followed for 1 to 2 weeks post-challenge and survival was expressed as a percentage of the total number of animals (10 mice/group).

6.Attached Figures 1A to 1D show the protection achieved by passive immunization with the hyperimmune rabbit sera. Figure 1A shows the results of challenge with 1 x 10^7 cfu of C388/90 (serotype Ia). Mice immunized with the gbs2018 hyperimmune sera had approximately an 80% survival rate 14 days post challenge, which was better than the approximately 60% survival rate with the positive control Sip sera. Mice immunized with PBS had only about a 10% survival rate 14 days post challenge. Figure 1B shows the results of challenge with 5 x 10^6 cfu of ATCC12401 (serotype Ib). Mice immunized with the gbs2018 hyperimmune sera had approximately a 40% survival rate 14 days post challenge, whereas mice immunized with the

positive control Sip sera or PBS had survival rates of below 10% 14 days post challenge. Figure 1C shows the results of challenge with 1 x 10^8 cfu of ATCC12403 (serotype III). Mice immunized with the gbs2018 hyperimmune sera had approximately a 70% survival rate 11 days post challenge, which was better than the survival rates observed for mice immunized with the positive control Sip sera (~ 50%) or PBS (~ 10%) at 11 days post challenge. Figure 1D shows the results of challenge with 1 x 10^8 cfu of ATCC49447 (serotype V). Mice immunized with the gbs2018 hyperimmune sera had approximately a 55% survival rate 14 days post challenge, which was better than the survival rates observed for mice immunized with the positive control Sip sera (~ 20%) or PBS (~ 10%) at 14 days post challenge.

7.For active immunization studies, CD-1 female mice (6-8 weeks) were immunized with 25 μg of the recombinant gbs2018 or gbs0031 antigens described above. Animals were pre-screened for pre-existing GBS-specific antibodies by testing their sera with ELISA and only animals without a significant reaction were used in the study. The antigens were injected subcutaneously into the mice, adjuvanted with Complete Freund adjuvant (CFA) or 1% ALUM. Animals were boosted twice with the same amount of antigen and Incomplete Freund adjuvant (IFA) at days 14 and 28. Mice immunized with PBS and adjuvant served as negative controls. Mice immunized with gbs0031 or *S. agalactiae* lysate from the strain with which the mice were subsequently challenged served as positive controls. Freshly grown *S. agalactiae* strains ATCC12401 (serotype Ib) and ATCC49447 (serotype V) were used for the animal challenge studies. In order to determine the viable cell numbers present in the bacterial inoculum, colony forming units (cfus) were determined by plating on blood agar plates. 10^6 or 10^8 cfus were applied intraperitoneally into mice. Protection was measured by a lethal sepsis model, where survival rates were followed for 10-11 days post-challenge and survival was expressed as a percentage of the total number of animals (10 mice/group).

8.Attached Figures 2A and 2B show the protection achieved by active immunization. Figure 2A shows the results of challenge with 1 x 10^6 cfu of ATCC12401 (serotype Ib). Mice immunized with the gbs2018 antigen had approximately a 50% survival rate 10 days post challenge, whereas the negative control mice had a survival rate of about 30% 10 days post challenge. Mice immunized with the positive control Sip or lysate had survival rates of about 65% and 90%, respectively, 10 days post challenge. Figure 2B shows the results of challenge with 1 x 10^8 cfu of ATCC49447 (serotype V). Mice immunized with the gbs2018 antigen had approximately a 50% survival rate 11 days post challenge, which was better than the survival rates observed for mice immunized with the positive and negative control groups at the same time point.

9.In conclusion, the active and passive immunization studies demonstrate that a gbs2018 antigen corresponding to amino acids 36 to 612 of SEQ ID NO: 364 produces a protective immune response against challenge with *S. agalactiae*.

10. I declare that all statements made of my knowledge are true and all statements made on the information are believed to be true; and, further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereupon.

Date: 11-MAY-2007

B. Senn

Beatrice Senn, Ph.D.

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Curriculum Vitae

Name	Beatrice Senn
Work address	Intercell AG Campus Vienna Biocenter 6 A-1030 Wien
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Home address	Gumpendorferstrasse 135/41 A-1060 Wien
Birth date	December 5, 1971
Nationality	Swiss
Marital status	single
Languages	German, English, French

Education

1978 – 1984	Primarschule, Ebmatingen (ZH)
1984 – 1986	Sekundarschule, Maur (ZH)
1986 – 1990	Handelsmittelschule, Zürich Hottingen
1990 – 1992	Gymnasium Typus E (Wirtschaft), Kantonsschule Zürich Enge
1992 – 1997	Studies in biochemistry, molecular biology, cell biology, genetics and immunology (Abt. XA) Swiss Federal Institute of Technology ETH, Zürich
1996	Diploma thesis at the Institute of Experimental Immunology under the supervision of Dr. P. Seiler and Prof. Dr. H. Hengartner Subject: Generation and characterization of lymphocytic choriomeningitis virus antibody escape variants
1997 – 2002	Ph.D. thesis at the Institute of Experimental Immunology under the supervision of Prof. Dr. H. Hengartner and Prof. Dr. R.M. Zinkernagel Subject: Analysis of anti vesicular stomatitis virus B cell responses in immunoglobulin transgenic and immunoglobulin knock-in mice
2002 – 2004	Post Doc at the Institute of Experimental Immunology under the supervision of Prof. Dr. H. Hengartner and Prof. Dr. R.M. Zinkernagel Subject: Further analysis of the anti-vesicular stomtatis virus immunoglobulin knock-in mice

2004 – 2005 Senior staff scientist in the Pharmacology/Toxicology department at Intercell AG, responsible for the animal models

2005 - Head of Infectious Disease Models at Intercell AG, responsible for the animal models and for the animal facility

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Ciurea A, Klenerman P, Hunziker L, Horvath E, Senn BM, Ochsenbein AF, Hengartner H, Zinkernagel RM.Viral persistence in vivo through selection of neutralizing antibody-escape variants. Proc Natl Acad Sci U S A. 2000 Mar 14;97(6):2749-54.

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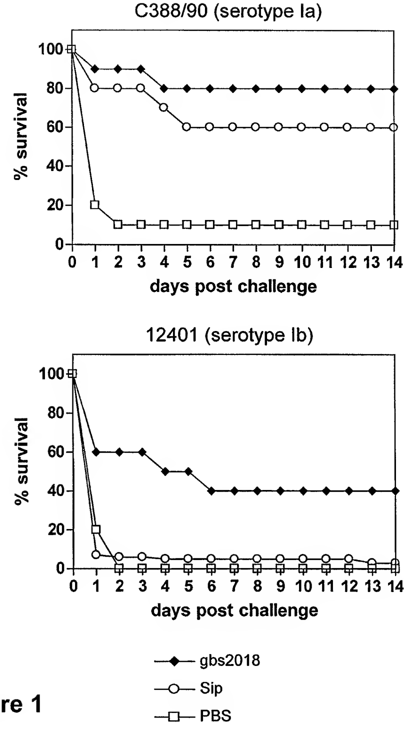
choriomeningitis virus carrier mice expressing a transgenic virus-neutralizing antibody. J Immunol. 1999 Apr 15;162(8):4536-41.

* both authors contributed equally to this work

A

D



C388/90 (serotype Ia)
12401 (serotype Ib)
% survival
days post challenge
0
20
40
60
80
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
gbs2018
Sip
PBS

Figure 1

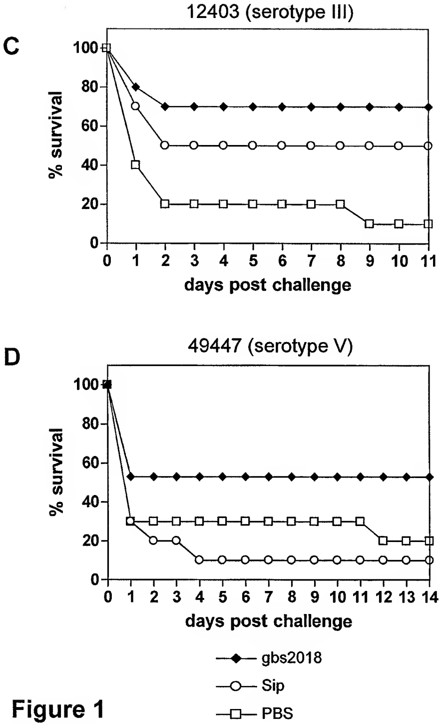

C
12403 (serotype III)
% survival
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11
days post challenge
D
49447 (serotype V)
% survival
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
days post challenge
gbs2018
Sip
PBS

Figure 1

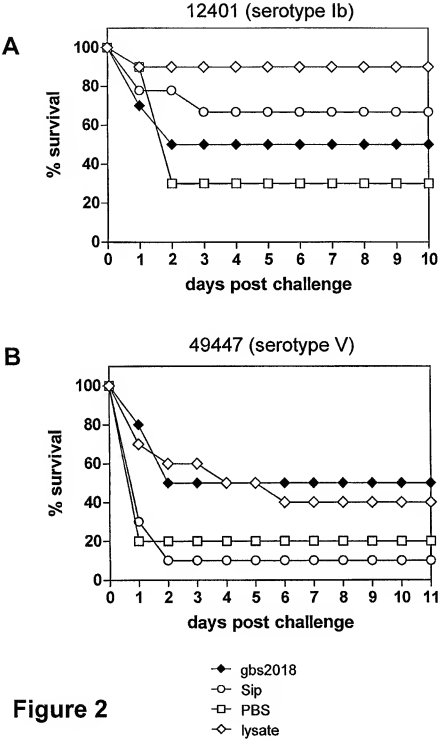

A
12401 (serotype Ib)
% survival
days post challenge
B
49447 (serotype V)
% survival
days post challenge
gbs2018
Sip
PBS
lysate

Figure 2